Selenastrum capricornutum 96-hour Chronic Toxicity Test

1.0 OBJECTIVE

In laboratory tests designed to determine the toxicity of low salinity water samples, the unicellular alga *Selenastrum capricornutum* is exposed to ambient samples for 96 hours, after which the cell growth is determined in each sample. Observed effects may be related to the presence of contaminants or to naturally occurring factors. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as other water quality parameters, such as pH, dissolved oxygen, total dissolved solids, hardness, salinity, temperature, ammonia and conductivity.

In this procedure, water samples collected from field stations are divided into replicate flasks in the laboratory. A known cell density of *Selenastrum* is placed into each replicate container and monitored for growth. Because the test measures effects on an early life-stage of an ecologically important species possessing relatively stringent water quality requirements, the results constitute a good basis for decisions concerning either hazard evaluation or the suitability of estuarine waters for aquatic life (US EPA 1994).

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (SOP).

2.1 Culture

- Airstones and culture air system
- 2-liter flasks or similar volume containers
- 4:1 water prepared from Perrier® or Evian® and distilled water (25 ± 1°C.)

2.2 Test Initiation

- 125-ml Erlenmeyer flasks (3-5 per sample concentration)
- 1000-mL volumetric flasks (2) and pipettes for reference toxicant dilutions
- Water bath or environmental chamber
- Data sheets
- Gloves and appropriate safety gear (see MPSL lab safety manual)
- Sample vials for reference toxicant analysis (new polyethylene 30 ml, acid washed)
- Auto-pipettes
- Analytical balance
- Plastic squirt bottles

2.3 Water Quality

- Meters and probes for measuring pH, dissolved oxygen, hardness, ammonia, and conductivity
- Thermometers (glass mercury thermometer and continuously recording thermometer)
- Graduated pipettes (10-ml) and hand pipette pump for water quality sampling
- Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 DILUTION WATER

In every step of this procedure, use Granite Canyon Nanopure® water mixed with Evian® in a 4:1 ratio. Culture water and samples should also be amended with nutrient solutions (see below).

3.0 EXPERIMENTAL DESIGN

Aquatic toxicity tests can be used as screening tools or as part of more comprehensive studies to assess water quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, and comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of four replicate flasks for each sample concentration. The quality of test organisms and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and control water (negative controls). Testing of reference sites or receiving water is recommended to demonstrate the suitability of test sites in the absence of toxic contaminant concentrations. Test conditions of temperature and photoperiod are controlled as indicated below, and pH, NH_3, conductivity, and dissolved oxygen are measured at the beginning and end of the exposure. Temperature is measured continuously, and hardness is measured at the beginning of the test.

4.0 PREPARATION OF SAMPLES FOR TESTING

Because of the short holding time, tests will generally be initiated on the same day as sample receipt. Place appropriate sample volume in the constant temperature room. Allow particulates to settle out and oxygen concentrations to equilibrate below super-saturated levels. Add one mL of each of 5 nutrient solutions per one liter of sample or control water. Recipes for nutrient solutions are in EPA 1994.

5.0 CONTROLS

5.1 Dilution Control

Dilution control consists of 4:1 culture water that has received a nutrient boost.

5.2 Reference Toxicant Tests

A reference toxicant test must be conducted concurrently with every test to indicate the sensitivity of the organisms and the suitability of the test methodology. Reagent grade copper chloride ($CuCl_2$) should be used as the reference toxicant for *Selenastrum* tests, unless another toxicant is specified by the Regional Water Quality Control Board or other appropriate regulatory agency. Prepare a 10,000 μg/liter $CuCl_2$ stock solution by adding 0.0268g reagent grade $CuCl_2$ to one liter of distilled water in a volumetric flask. Cap tightly and mix thoroughly. Sample the reference toxicant stock solution at the beginning of the test for chemical verification of the copper concentration. Acidify samples for analysis in clean sample vials with 1% by volume 14N-reagent grade nitric acid.

Reference toxicant solutions should be four replicates of 0 (control) 18, 32, 56, 100, and 180 μg/liter. Other concentrations may be added between these if greater precision is desired for quality control chart purposes. Prepare concentrations according to dilution schedule. Start with the control solutions and progress to the highest concentration to minimize contamination.

6.0 TEST ORGANISMS

6.1 Culturing *Selenastrum*

If Selenastrum test starters are not purchased from a supplier, then laboratory cultures can be used. Cultures are prepared from a starter culture by adding starter cells to nutrient-boosted dilution water. Prepare dilution water by adding one mL of each nutrient solution per one liter of 4:1 water. Mix thoroughly and vacuum filter through a 0.22 μm filter. Maintain this culture at 25°C under fluorescent light of 86 ± 8.6 $\mu E/m^2/s$. Transfer one to two mL of stock culture weekly to 50 – 100 mL of new culture medium to maintain a continuous supply of cells.

6.2 Inoculating Test

Prepare cell inoculum by determining the cell density of the stock culture. Using the test initiation data sheet, follow the equations for determining the volume of inoculum. The final concentration of cells in the test flasks should be 10,000 cells/mL. Measure temperature, dissolved oxygen, pH, ammonia, alkalinity, hardness, and conductivity in each sample at the beginning and end of the test. Sample the initial test solutions at the time of dilution preparation.

7.0 DAILY TEST MONITORING

Flasks should be shaken twice a day in the morning and afternoon. If a test is to be conducted over a weekend then flasks should be shaken at the start and end of the work shift.

8.0 TERMINATING THE TOXICITY TEST

After 96 hours of exposure the cell density in each flask is determined using hemacytometer or UV spectrophotometer. Final water quality must be sampled at the termination of the test. Take the completed data sheet to the office for data entry and analysis. Notify the data analyst that the data has arrived. Make sure the data sheets are placed in the proper location and that the person keeping track of the data knows where it is.

9.0 DATA HANDLING AND TEST ACCEPTABILITY

Immediately after test termination, check the data sheet to determine whether dilution water and conductivity controls have acceptable growth (mean of > 200,000 cells/mL). If not, notify the project officer without delay.

This toxicity test procedure is considered acceptable if *Selenastrum* growth in controls is greater than or equal to 200,000 cells/mL. Variability among control replicates must not exceed 20%. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures range from 25 ± 1°C; acceptable dissolved oxygen concentration is 60-100% saturation.

10.0 REFERENCES

U.S. Environmental Protection Agency. 1994. Short-term methods for estimating the chromic toxicity of effluents and receiving water to freshwater organisms. EPA-600-4-91-002. Office of Research and Development. Washington, DC.

11.0 TEST SUMMARY

Species:	*Selenastrum capricornutum*
Test Duration:	96 hours
Renewals:	None
Organism Source	In-house cultures or supplier
Age of test organisms:	4-7 days
Test Temperature:	25 ± 1°C
Light intensity:	Ambient laboratory illumination 10-20 µE/m^2/s
Photoperiod:	16 hour Light: 8 hour Dark
Replication:	4 replicates
Test Containers:	125-mL Erlenmeyer flasks
Test solution volume:	50-mL minimum

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Loading:	10,000 cells/mL
Water Quality:	pH, D.O. temp, conductivity, NH_3, alkalinity, hardness, a ammonia
Reference Toxicant:	Copper Chloride ($CuCl_2$)
Stock Solution:	0.0268 g in 1 liter of distilled water (= 10,000 mg/L).
Dilutions:	0, 18, 32, 56, 100, 180 μg/L
Daily Monitoring:	Shake 2 times
Safety:	Wear protective clothing; read applicable MSDS, be familiar with the lab safety manual prior to testing.
Quality Control:	Fill out all data sheets completely. Be familiar with QA Project Plan prior to testing.
Acceptability Criteria:	Dilution Controls: >200,000 cells/mL Variability among control replicates <20%
Temperature range:	24° to 26°C.
Dissolved oxygen:	maximum 9.67 mg/L